

Field evaluation of commercial formulations of the codling moth granulovirus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards

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Abstract

Inundative applications of the codling moth (CM), *Cydia pomonella* L., granulovirus (CpGV), which target neonate larvae before or during initial entry into fruit, offer potential for selective control of this key pest. In field tests on apple we compared the persistence and efficacy of single applications of three CpGV products approved for organic orchards in North America. In addition, the success of repeated (2–14) applications of one product (Cyd-X) as a principal control measure for CM in apple orchards was monitored following operational use by cooperating growers at four separate locations. In the first study, an early season application of all products at label rates remained highly effective for the first 24 h (averaging 94% larval mortality relative to controls) and moderately effective after 72 h (averaging 71% mortality) during dry sunny conditions. Significant activity remained up to 14 days, suggesting prolonged survival of the virus in UV-protected locations, such as the calyx of fruit. A second application later in the season was slightly less effective. Data obtained from commercial sites provide circumstantial evidence for the effectiveness of well-timed CpGV applications against CM outbreaks. In all cases where first generation larvae were targeted beginning at egg hatch (≈ 250 degree days) and treated areas monitored (0.3–1.6 ha plots), fruit damage during the second larval generation was reduced or eliminated. Based on the number of live larvae recovered throughout the season, mortality rates remained high (80.3–100% across sites). The cumulative number of moths caught in pheromone-baited traps was reduced (66–94%) in the second flight. Data from tree bands placed to catch diapause-destined larvae indicated overwintering generations remained low in treated sites (≤ 0.18 larvae/band). Published by Elsevier Inc.

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1. Introduction

With the exception of Japan and parts of mainland Asia, the codling moth (CM), *Cydia pomonella* L., remains a principal insect pest of apples, pears, and occasionally walnut throughout the temperate regions of the world (Barnes, 1991; Beers et al., 1993). CM larvae bore deep into the fruit making it unmarketable and may go through several generations in a growing season. Although mating disruption with synthetic lures has

been widely commercialized, its effectiveness declines at high pest pressure. The majority of conventional growers still rely on chemical insecticides to maintain this pest at economically acceptable levels (Beers et al., 1993; Calkins, 1998; Vickers and Rothschild, 1991). Such practices are incompatible with environmental goals and may jeopardize worker safety (Lacey and Shapiro-Ilan, 2003). The implementation of the 1996 Food Quality Protection Act in the US and anticipated loss of registered compounds such as Guthion (azinphos-methyl), coupled with recent documentation of direct and cross-resistance to a range of commonly used compounds (Dunley and Welter, 2000; Sauphanor et al., 2000; Varela et al., 1993)

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also limits the insecticidal options for controlling CM larvae.

Although historically ‘soft’ control options have been limited, the granulovirus of *C. pomonella* (CpGV) has received extensive interest as an inundative microbial control agent. The virus is normally applied as an aqueous suspension targeted at neonate larvae which ingest occlusion bodies (granules) before or during initial entry into fruit. After ingesting the virus, the granules quickly dissolve in the alkaline midgut releasing the virions, which pass through the peritrophic membrane to establish a transient infection before invading most of the major body tissues including the tracheal matrix, epidermis, and fat body (Federici, 1997). Host death and liquefaction typically occur in 5–10 days. Over the past 30 years, numerous field trials have demonstrated good activity of CpGV in a variety of settings across Europe, South Africa, Australia, New Zealand, South America, and North America (Falcon and Huber, 1991; Guillon and Biache, 1995; Huber, 1986; Jaques, 1990; Vail et al., 1991). CpGV’s specificity for CM and some closely related species and safety to non-target organisms have been thoroughly documented (Gröner, 1986), thus it may contribute significantly to the conservation of non-target predaceous and parasitic arthropods that suppress secondary pests in the orchard.

Despite its promise, the commercial development and adoption of CpGV has been limited (Cross et al., 1999). Concerns expressed by growers are: the increased number of shallow stings to the fruit, short residual activity and need for multiple applications, slow speed of kill, lower efficacy against high density codling moth populations, and expense and quality control of the virus product (Glen and Clark, 1985; Jaques et al., 1987). However, several newly formulated products on the market have been registered in North America and approved for use in organic orchards. We report on field-based studies comparing the persistence and efficacy of individual applications of three CpGV products. In addition, the success of repeated applications of one product as a principle control measure for CM was monitored following operational use by cooperating growers at four separate locations in the Pacific Northwest in 2003.

2. Materials and methods

2.1. Comparison of CpGV formulations

2.1.1. Study site

This study was conducted at the USDA experimental orchard near Moxee, WA, USA. Treatments were applied within a 0.5 ha plot of Delicious (strain Red Chief) planted on EMLA 7 rootstock in 1998. Trees were ≈ 2 m high with spacing of 3.7 m in rows and 5.5 m between rows, excluding Manchurian and crab apple snowdrift pollinators. Trees were maintained according to normal horticultural practices for the region (J. Gefre, personal communication) although no insecticides were used in 2003 other than experimental treatments.

2.1.2. Virus applications

Applications of CpGV were made between 6 and 11 a.m. on 2 June and again on 14 July 2003 using a Stihl SR420 motorized backpack air-blast sprayer. The backpack air-blast sprayer was chosen to provide coverage similar to a tractor-pulled air-blast sprayer (Lacey et al., 2000). Spray dates were shortly before the start of egg hatch for first and second CM generation (177 and 927 degree days (DD) postbiofix, respectively. Biofix was taken as the first consistent flight of male CM monitored from pheromone-baited traps, to predict peak egg hatch). Developmental rates and thresholds (≈ 7 – 31 °C) followed the Washington State University model (Beers et al., 1993). These commercial products were applied in a complete randomized block design to individual tree plots with 10 trees per treatment (Table 1). Formulations were prepared with fresh refrigerated product the morning of use. The volume application rate was 935 L ha^{-1} (100 gal ac^{-1}) and the spreader-sticker NuFilm-17 (Miller Chemicals and Fertilizer; Hanover, PA) was added to all formulations at 0.44 L ha^{-1} (6 oz ac^{-1}). To promote coverage, trees were sprayed from multiple angles and a three-sided moveable tarpaulin screen (each side 3×3 m) held by four assistants was used to prevent overspray or drift of treatments. Control trees were sprayed with water and NuFilm-17 only and, for the second application, were selected from a single row upwind of virus treatments. Shade temperature, relative humidity and solar intensity in an exposed location were

Table 1
CpGV applications made June and July 2003 to experimental plots at Moxee, WA

Product ^a	Manufacturer	Granules L^{-1} ($\times 10^{13}$) ^b	Label rate; L ha^{-1} (oz ac^{-1})	Low rate; L ha^{-1} (oz ac^{-1})	High rate; L ha^{-1} (oz ac^{-1})
Cyd-X	Certis, USA	3	0.07–0.44 (1–6)	0.22 (3)	0.44 (6)
Virosoft	Biotepp	4.2	0.23 (3.2)	0.23 (3.2)	0.44 (6)
Carpovirusine	Sumitomo	≥ 1	1 (13.7)	0.44 (6)	1 (13.7)

Control trees were treated with water and NuFilm-17 only.

^a Volume application rate 935 L ha^{-1} ; NuFilm-17 spreader-sticker included at 0.44 L ha^{-1} .

^b Based on label counts.

monitored every 30 min using a Hobo H8 Pro Series (Onset; Pocasset, MA) and LI-400 datalogger fitted with a pyranometer (Li-Cor; Lincoln, NE).

2.1.3. Assessments

For the assessments immediately after spraying (0 day) and at 1-, 3-, 7-, 10-, and 14-day intervals fifty pest-free apples per treatment (five per tree) were returned to the laboratory to bioassay for residual activity. On each sample day, all fruit was removed by 8 a.m. and exposed to neonate CM the same morning. The 1- and 14-day samples were excluded in the July study. Because the virus may degrade more rapidly in exposed locations, fruit were selected from different heights and orientations to obtain a representative sample for each tree. To protect spray residues handling was minimized. Apples were placed in an individual cup with a tightly fitting lid on site. In the first study fruit was held in place with an upturned tack inserted through the bottom of a 512-oz Portion cup (Sweetheart; Owings Mills, MD). In the second set of samples, 12-oz cups (Styro-Cup; Win-Cup; Phoenix, AZ) were required for the larger fruit. In this case fruit were suspended by the stem, which prevented larvae entering via the tack hole. The stem was threaded through a hole cut in the lid and held with a binding clip, such that the fruit did not touch the inside of the cup.

The upper surface of each apple was infested with five neonates obtained from a colony of CM maintained on an artificial diet (Toba and Howell, 1991) at the USDA-ARS Yakima Agricultural Research Laboratory. Neonates hatched from eggs laid on wax paper and were held at 27°C before use. Only neonates that hatched the same morning were used. Apples were incubated at 25°C

and destructively sampled to quantify fruit damage and larval survivorship 10 days postinfestation. The vast majority (>95%) of CpGV-killed larvae died as first instars and were identifiable by their milky-white color and tendency for the epidermis to rupture when prodded. For the July application the size (diameter and depth) of stings were recorded from a sub-sample of fruit ($n=350$) using a modification to the method of Glen and Payne (1984). For each injury, surface diameter was categorized; A (0–2 mm), B (2–4 mm) or C (>4 mm) and depth into fruit was categorized; A (0–2 mm), B (2–3 mm) or C (>3 mm).

2.2. Evaluations at commercial orchards

2.2.1. Study sites

The success of one CpGV product (Cyd-X, Certis, USA) was evaluated as a primary control measure for CM following operational use in a full season program by cooperating growers. Fruit injury and CM population dynamics were monitored in six plots at four separate commercial organic orchards encompassing discrete regional differences (Table 2). Historically all sites had suffered repeated damage from CM. Treatments and associated monitoring were generally confined to ‘hot-spots’ (identified by the growers based on localized 2002 infestations) where mating disruptors (200–400/ac) and some sanitation of damaged fruit was also employed.

2.2.2. Virus applications

Individual growers applied Cyd-X within manufacturer’s label recommendations; i.e., 220–437 ml product at a volume application rate of 935–1870 L ha⁻¹ (3–6 oz

Table 2

Codling moth control with CpGV (Cyd-X, Certis, USA) at organic orchards in Washington State, USA; details of sites and treatments

Region (WA)	Treated area monitored		Application frequency (and timing in degree day intervals) ^a
Quincy ^b	Braeburn (1 ha trellised; planted 1999 at 3532 trees/ha)	First generation	1 (251)
		Second generation	1 (1135)
Royal City ^c	Braeburn (1.6 ha; planted 1992 at 742 trees/ha)	First generation	5 (134, 245, 353, 495, 667)
		Second generation	4 (1102, 1280, 1833, 2404)
Parker Hgts.	Gala (1.4 ha trellised; planted 1991–1995 at 5980 trees/ha)	First generation	6 (260, 385, 530, 668, 793, 935)
		Second generation	6 (1252, 1428, 1632, 1843, 2010, 2177)
	Golden Delicious (1.2 ha; planted 1968 at 266 trees/ha)	First generation	6 (260, 385, 530, 668, 793, 935)
		Second generation	8 (1252, 1428, 1632, 1843, 2010, 2177, 2341, 2476)
Mattawa ^d	Delicious (1 ha; planted 1979 at 424 trees/ha)	First generation	2 (660, 1085)
		Second generation	5 (1290, 1470, 1660, 1865, 2010)
	Granny Smith (0.3 ha; planted 1979 at 424 trees/ha)	First generation	2 (660, 1085)
		Second generation	5 (1290, 1470, 1660, 1865, 2010)

^a Cyd-X applied within label recommendations (see Section 2).

^b Summer oil (0, 192 DD), Bt (Deliver) (28 DD for leafrollers), and Entrust (spinosad) (284 DD).

^c Summer oil (134, 245, 495, 667 DD), Entrust (667 DD).

^d Both varieties; summer oil (253 DD), Entrust + summer oil (351, 509, 660 DD), and Bt (694, 862 DD); Carpovirusine (not Cyd-X) applied at 1290 and 1660 DD.

in 100–200 g ac⁻¹). The spreader-sticker NuFilm-17 was included at 0.58–1.17 L ha⁻¹ (0.5–1 pint ac⁻¹). All virus applications were made using a conventional tractor-pulled air-blast sprayer fitted with a fan and adjustable hydraulic nozzles. The airflow, operating pressure, selection of nozzle orifice diameters and nozzle direction was designed to blow the spray through the canopy typically delivering $\approx 85\%$ of the spray volume to the top two thirds of the tree. Applications were timed according to routine monitoring practices utilizing the number of day degrees (DD) accumulated at each location since biofix (as previously defined). The number of applications also reflected the number of moths caught. Details were checked against grower spray records throughout the season. In addition to Cyd-X, oil applications were made in some locations (primarily as an early season ovicide) and other insecticide treatments applied against other pests such as leafrollers, as listed in Table 2 footnotes.

2.2.3. Assessments

Repeated sampling was used to estimate the extent of CM fruit injury in treated areas. All damaged fruit on sampled trees was returned to the laboratory to assess larval mortality resulting from treatments (typically indicated by shallow stings and dead or missing larvae). A minimum of 30 trees (100 trees in high density plantings) were randomly selected for monitoring towards or shortly after the end of the first (711–1194 DD) and second (1824–2632 DD) CM generations; representing ≥ 88 and $\geq 83\%$ egg hatch, respectively. Any additional fruit removed as a result of routine worker sanitation were obtained and included in the assessments of larval mortality. At Mattawa, fruit damage during the first larval generation was not assessed.

The cumulative number of moths caught in pheromone-baited ‘delta’ monitoring traps located within treated plots was recorded at weekly intervals throughout the season. Up to eight traps were used in each location. Traps containing kairomone lures which also caught females (Knight et al., 2002) were included in two locations (Quincy and Parker Hgts.). The locations of traps were not changed between flights, although lures were replaced as required. First flight was taken as ≤ 840 DD and second flight as > 840 DD (Beers et al., 1993).

Corrugated cardboard bands were tied around the circumference of the trunk of selected trees (30–46 cm above the ground) to capture mature diapause-destined larvae searching for overwintering cocooning sites. Trees selected for banding were not previously used for fruit damage assessments. The cardboard used to make the bands (8-cm wide single faced flute size B; Xpedx; Portland, OR) was first folded flute-to-flute to encourage larvae to spin-up within the double layer of cardboard to aid removal (J. Upton, personal communication). A minimum of 81 trees per location were banded (one band per tree) from 13th to 21st August (1755–2076

DD). Bands were removed at harvest (2136–3100 DD depending on the apple variety) and examined in the laboratory for cocooning CM. Four untreated sites were assessed concurrently with virus-treated plots. Between 55 and 168 trees were banded in crab apples and untreated commercial plots in Yakima, Parker Hgts., and Moxee (two sites).

2.3. Data analysis

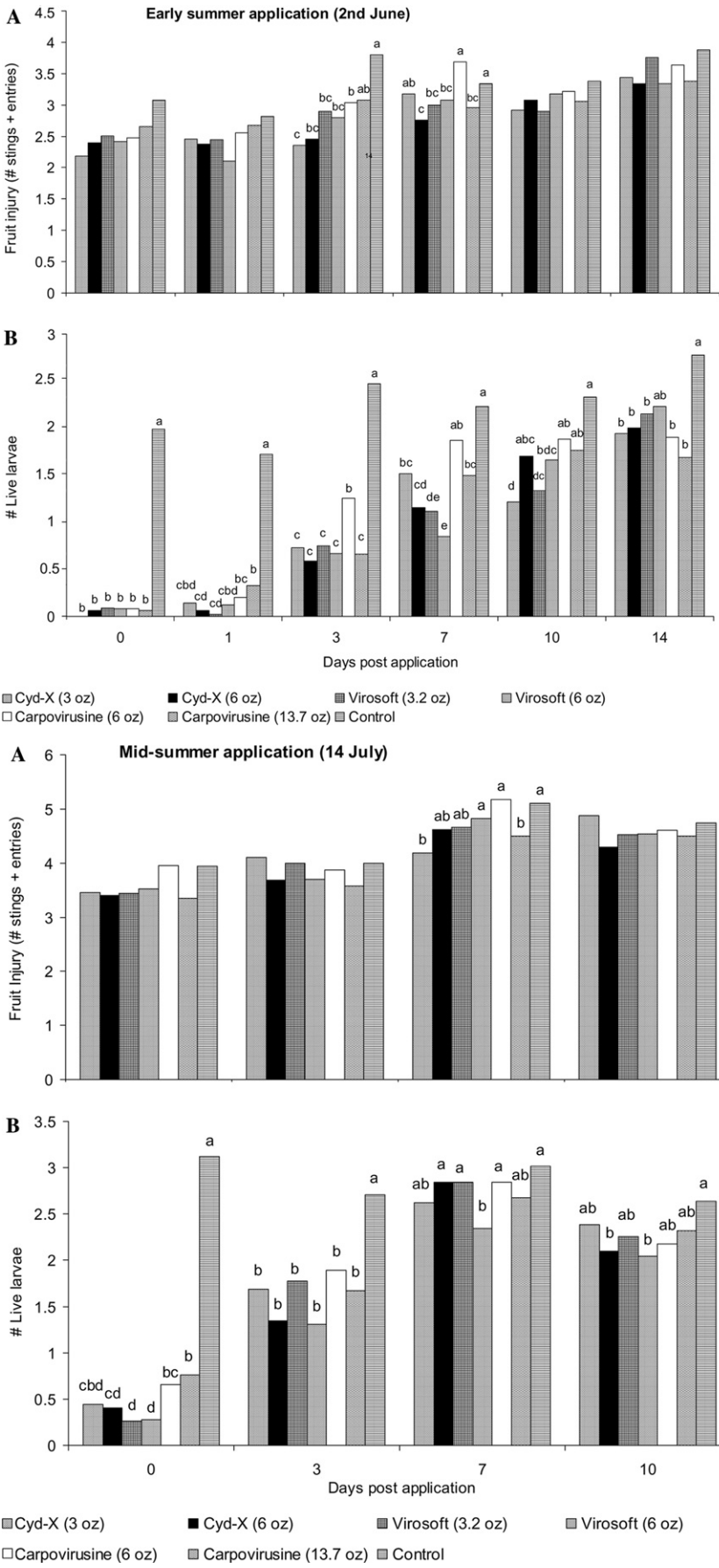
For the product comparison study, treatment effects on fruit damage and larval survival were compared for each test using repeated measures multivariate ANOVA following a $\log(n+1)$ transformation of data. To identify trends further comparisons were made for each sample date using univariate ANOVA. Significant means in all ANOVAs were separated with Fisher’s LSD for multiple comparisons, $P < 0.05$. Additionally each product was compared at recommended label rates and the effect of dose was compared for each product (planned comparisons). Each replicate was based on the overall mean for each tree for a treatment sample size of 10. Data on size of fruit injury (three diameter and depth classifications) were obtained in the second test. Comparisons of the type of injury occurring on fresh CpGV-treated and untreated fruit were made using χ^2 tests for independence.

For the grower assessments, data on fruit injury and CM bionomics were compared between first and second generation samples for a given site. Data were not compared statistically between different sites due to differences in infestation levels and treatments applied. Because the number of clean fruit per tree was not assessed, fruit injury was expressed as the proportion of infested fruit per sampled tree and compared with χ^2 tests in paired-comparison contingency tables. The number of injuries per fruit and larval mortality (determined in laboratory assessments which included any samples obtained following grower sanitation) were compared using similar procedures. The cumulative number of moths caught during the first and second flights was compared using paired-samples (non-independent) t tests using each trap location to pair the sample following $\log_{10}(n+1)$ data transformation. Comparisons could not be made at one location with a single trap. Because no trees were banded at the end of the first generation, no comparisons were made for mature larvae.

3. Results

3.1. Comparison of CpGV formulations

Fruit injury and number of live larvae recovered from fruit following different CpGV treatments are shown together for two separate tests in Figs. 1A and B. Multi-



variate tests showed no significant treatment by time interaction for overall fruit injury (defined as the number of shallow stings and deep entries combined) following both June and July applications ($F_{30,202} = 1.1$, $P = 0.33$; $F_{18,148} = 0.94$, $P = 0.53$, respectively). By contrast for larval survival, the treatment by sampling date interaction was highly significant (June, $F_{30,202} = 6.2$, $P < 0.0001$; July, $F_{18,148} = 5.6$, $P < 0.0001$).

For overall fruit injury, no differences between treatments were observed (one-way ANOVA for each date) with the exception of the day 3 and 7 (June) and day 7 (July) sample where statistically fewer injuries were observed in some CpGV-treated samples compared with untreated fruit (Fig. 1A). Among fruit treated with CpGV, planned contrasts revealed no difference in overall fruit injury between any products at label rates, or between doses for a given product, with a single exception for Carpovirusine dose in the second (July) study ($F = 7.51$, $P < 0.01$). Although in both tests the overall number of injuries remained high, the damage in CpGV-treated fruit was clearly reduced as assessed by both diameter and depth of larval entries (χ^2 , $P < 0.001$, $df = 2$). For example, while 97.6% of the larvae on untreated fruit formed deep entries, 69% of damage on CpGV-treated fruit was in the form of shallow 'stings' < 3 mm deep (Fig. 2).

For the June applications, the residue on fruit for all products used at label rates remained highly effective for the first 24 h (averaging 94% larval mortality relative to controls) and moderately effective (71%) after 72 h (Fig. 1B). Mortality remained statistically but not economically significant after 14 days in five out of six treatments. The residue on fruit from the July application had a similar effect on larval survival but overall persistence was reduced. Only two out of six treatments registered significant activity after 10 days.

A simple linear model was used to estimate half-lives at label rates (days until 50% of initial activity based on decline in larval mortality with time). June applications provided the following: Cyd-X (3 oz ac^{-1}) = 8.3 days ($R^2 = 0.81$); Virosoft (3.2 oz ac^{-1}) = 8.7 days ($R^2 = 0.95$); and Carpovirusine (13.7 oz ac^{-1}) = 7.9 days ($R^2 = 0.75$). July applications (same rates); Cyd-X = 4.2 days ($R^2 = 0.86$); Virosoft = 3.8 days ($R^2 = 0.76$); and Carpovirusine = 4.5 days ($R^2 = 0.87$). Planned contrasts revealed few differences in larval mortality between any products applied at label rates or between doses for a given product with the following exceptions. For product, Virosoft $>$ Carpovirusine on day 1 (June) and day 0 (July). For dose applied, Carpovirusine day 3 (June), Cyd-X day 10 (June), and Virosoft day 7 (July).

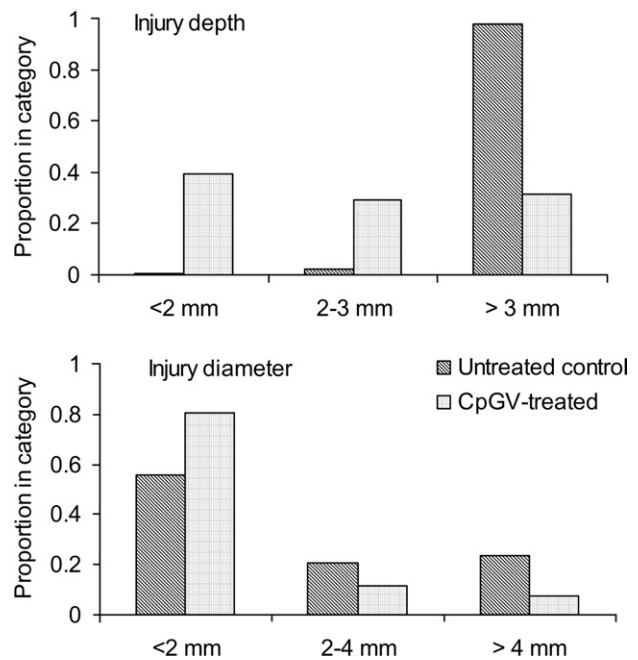


Fig. 2. Codling moth fruit injury categorized by size of larval entries. Data compare fruit treated with CpGV and untreated controls [$n = 350$ fruit (1217 injuries)]. Virus-treated fruit were pooled across six virus treatments (see Section 2) and exposed to larvae the same day as application.

Conditions remained sunny and no rain fell throughout the study periods. However, it was noticeably hotter and drier following the second application with maximum daytime temperatures in the range 30–38 °C as opposed to 22–32 °C following the first application. Maximum solar radiation intensity remained similar ($\approx 1000 \text{ W m}^{-2}$) throughout both studies (data not shown).

3.2. Evaluations at commercial orchards

Data obtained from commercial sites provide circumstantial evidence for the effectiveness of well-timed CpGV applications against codling moth outbreaks (Table 3). In plots where CpGV was used against first generation CM larvae, the proportion of fruit damaged the second generation was reduced or eliminated, with the reduction significant in three out of four cases. There were no differences in the number of injuries in fruit damaged in the first and second CM generations. Based on the high proportion of failed entries or shallow stings, the majority of neonates were presumed to have been killed (80.3–100% across all sites). Although most larvae were missing, those recovered were predominantly first instars with patent viral infections consistent in CpGV.

Fig. 1. (A) Fruit injury and (B) number of live larvae recovered from apples treated with CpGV. Data compare two doses for each of three CpGV products applied in an experimental orchard. Fruit was challenged with five-neonate larvae at various intervals after spraying. Values are based on nested means for 10 trees with five fruit removed per tree. Data shown for two separate tests; early summer and mid-summer. Letters above bars indicate differences between treatments for a given date following a significant one-way ANOVA for that date; $P < 0.05$, Fisher's LSD $\log(n + 1)$.

Table 3

Fruit injury and codling moth population dynamics monitored in organic apple orchards in Washington State, USA following various CpGV (Cyd-X, Certis USA) applications in 2003

Region (WA)	Cultivar		Damaged fruit/tree ^a	Injuries/fruit ^a	Proportion live larvae ^a	Adults/trap ^b	Larvae/tree band ^c
Quincy	Braeburn	First generation	0.04a (150)	1 (8)	0	4.8a (5)	n/a
		Second generation	0 b (100)	n/a	n/a	1.6a (5)	0 (153)
Royal City	Braeburn	First generation	0.76a (100)	1.06a (908)	0.03a	29a (4)	n/a
		Second generation	0.21 b (100)	1a (21)	0a	1.8 b (4)	0.01 (196)
Parker Hgts.	Gala	First generation	0.08a (300)	1.12a (654)	0.13a	20.3a (3)	n/a
		Second generation	0.03 b (180)	1.08a (263)	0.19 b	2.7 b (3)	0.005 (211)
	Golden Delicious	First generation	3.8a (30)	1.21a (114)	0.17a	27.1a (8)	n/a
		Second generation	2.5a (30)	1.2a (1135)	0.14a	5.3 b (4)	0.04 (160)
Mattawa	Delicious	First generation	n/a	n/a	n/a	18.3a (3)	n/a
		Second generation	0.81 (30)	1.19 (176)	0.14	6.3a (3)	0.11 (81)
	Granny Smith	First generation	n/a	n/a	n/a	21 (1)	n/a
		Second generation	1.9 (30)	1.21 (247)	0.08	2 (1)	0.18 (92)

^a Letters represent differences between samples around the end of the first and second CM generations (711–1194 and 1824–2632 DD). ($P < 0.05$, χ^2 tests in paired-comparison contingency tables; sample number in parentheses.)

^b Letters represent differences between cumulative pheromone trap catches of first and second flights (≤ 840 and > 840 DD). ($P < 0.05$, paired-samples t test $\log_{10}(n + 1)$; number of traps in parentheses.)

^c Overwintering generation only captured; number of trees banded in parentheses.

The cumulative number of adults caught in pheromone traps was reduced by 66–94% in the second flight, with this reduction significant in three out of five comparisons. Data from trap bands placed around trees to catch diapause-destined mature larvae indicated the extent of overwintering generations in virus-treated sites remained low (≤ 0.18 larvae/band). The CM larval parasitoid *Mastrus ridibundus* Gravenhorst (Hymenoptera: Ichneumonidae) was noted at two virus-treated sites. Wasp pupae were recovered from six out of 28 larvae at Mattawa and three of seven larvae at Parker Hgts. (representing 21 and 43% parasitism, respectively). At Mattawa, the wasp was not recovered from a larger sample (72 CM larvae recovered in 110 bands) at an adjacent non-organic plot treated with Guthion. By comparison with the virus-treated plots, between 5.8 and 10.1 CM larvae/band were recovered from the four untreated plots that were banded concurrently. *M. ridibundus* were found in two of the four untreated plots (accounting for 1.2–3.3% parasitism in these high density populations).

4. Discussion

The patterns of codling moth neonate mortality from the experimental orchard and organically managed orchards provide evidence for the value of CpGV in CM management strategies. The tests against field-aged residue confirmed the short-term efficacy of the three commercial CpGV products at label rates (Fig. 1B). Observations made at four commercial orchards were consistent with the effectiveness of repeated applications

timed to egg hatch of the first and second generations (Table 3).

4.1. Virus persistence

Although initial control (i.e., mortality from fresh residue) is comparable to many chemical pesticides, the shorter residual persistence of CpGV remains the major limitation for overall control. In the present study larvicidal activity declined to 50% of its original value after ≈ 8 days (early summer), although the decline was more rapid later in the season (≈ 4 days mid-summer). Although not specifically assessed virus activity may have been prolonged in UV-protected locations, such as the calyx of fruit. It should be noted that while in the assay larvae were placed directly on the fruit surface, in the field a majority of larvae may be infected prior to location of fruit. Studies in unmanaged orchards showed that 92% of eggs were oviposited onto leaves; with just 8% on the fruit itself (Jackson, 1979). In another study, only 6% of 3581 eggs were deposited on fruit (Summerland and Steiner, 1943). Using a treated leaf disc assay Ballard et al. (2000) showed that neonate larvae could acquire a lethal dose of CpGV in as little as 3.5 min, often before any significant feeding was observed. Thus, these estimates of virus persistence may be considered conservative. Previous studies have estimated CpGV half-lives at between 2 and 3 days (Glen and Payne, 1984; Huber, 1980; Jaques et al., 1987). However, differences in factors including formulation, method of application, and assessment make direct comparisons difficult.

The persistence of baculoviruses is determined by several factors, although on foliage exposure to solar radiation (particularly the damaging ultraviolet portion UV-B; 280–320 nm) is most critical (Jaques, 1985). In the morning and evening relatively less UV occurs than in the middle parts of the day (Ryer, 1997). Presumably more UV is reflected or absorbed in the atmosphere during early summer when the sun remains closer to either horizon. Thus, although overall solar intensity was similar, the greater contribution of UV-B at mid-summer may have contributed to the reduced persistence observed. The substantially higher mid-summer daytime temperatures ($>35^{\circ}\text{C}$) may have also been involved. Following laboratory tests with CpGV-treated apples, Keller (1973) reported neonate CM mortality was significantly reduced at 34°C , compared with studies in the range $15\text{--}30^{\circ}\text{C}$.

It is worth noting that because of the shallow dose-response curve, median lethal concentration (LC_{50}) of CpGV has been estimated at 32.7 capsules/ mm^2 on artificial medium (Lacey et al., 2002), the decline in larvicidal activity is unlikely to proportionally reflect virus inactivation. Glen and Payne (1984) describe a technique to measure inactivation of CpGV. Virus washed off the leaves of sprayed trees (at various intervals postspraying) is incorporated into an artificial diet and used for bioassay. The amount of infective virus remaining is calculated by comparing the sample mortality against a standard dilution series of known concentration. Using this approach in one trial Glen and Payne (1984) were able to show that although CpGV infectivity was reduced by half in 3 days, some activity persisted at least 4–8 weeks after spraying.

Despite the prolonged activity of virus in protected locations, to retain effective control CpGV must be reapplied throughout the peak egg hatch period. The recommended application intervals for the products tested range from 7 to 10 days. In the virus-treated plots in 2003, the predicted 5–95% egg hatch for CM (Beers et al., 1993) encompassed a 26- to 29- and 28- to 37-day period for the first and second generations, respectively, or 4–6 weekly applications per generation. Additional applications may be required in more southerly latitudes where a significant third CM generation is found, while fewer may be required in short growing seasons of northern Europe or Canada. For example, in Nova Scotia, where there is only one CM generation per year Jaques et al. (1994) reported that only two applications of CpGV were usually needed.

4.2. Commercial use

At commercial orchards where virus was used in a full season program, levels of CM fruit damage were considered acceptable for organic production. Although some additional control measures were used against the first

generation, applications of spinosad likely provided some early season control of CM at Mattawa, all growers considered CpGV to have played a key role in protecting their crop. Moreover, at one site (Parker Hgts. a small orchard combining young trellised Gala and 35-year-old Golden Delicious moderately infested with CM), CpGV was the only intervention throughout the whole season. Although some leafrollers were observed late season, no significant secondary pest problems occurred.

At an additional site (data not shown) a grower replaced weekly sprays of 1.5% summer oil with Cyd-X in approximately one-third of a 10 ha ‘Delicious’ orchard to combat the second generation of a very severe CM infestation. Although comparisons between the virus-treated area and the remaining portion (that was concurrently treated with five further applications of oil) suggested that the virus was the more effective at reducing the number of live larvae in fruit and tree bands, the grower still suffered complete loss of his crop due to fruit injury. This control failure emphasized the importance of maintaining early season control.

Although the slow speed of CpGV’s kill relative to chemical interventions remains a concern in terms of fruit damage (Fig. 1A) the majority of injuries were small and shallow (Fig. 2). While deep larval entries make fruit unmarketable (Beers et al., 1993), some packing houses may select fruit with shallow stings as ‘peelers’ suitable for applesauce or juice production, thus giving some economic return. Moreover, damage may not be as severe as Fig. 1A indicates for three reasons. First, as noted above, many larvae may become infected prior to location of fruit and thus our method of placing five larvae (representing high pressure) directly on the fruit surface might be a ‘worst case scenario.’ Second, as cell division occurs for four to five weeks in apples (longer for epidermal areas) (Tukey, 1981), some early season damage may ‘recover’ by harvest (although this has not been assessed). Finally, there is the potential to ‘selectively thin’ out early season damage by preferentially removing injured immature fruit during routine manual thinning practices. Thus, although CpGV is primarily aimed at population suppression, it appears fruit damage may be managed to acceptable levels.

In conclusion, our results suggest commercial CpGV formulations provide a valuable alternative for CM management. Weekly applications timed during peak egg hatch and integrated with other strategies such as mating disruption or other soft pesticides will likely provide effective population suppression for many growers with low to moderate codling moth pressure. The recovery of *M. ridibundus* at two sites is encouraging, suggesting that applications of CpGV are compatible with parasitoids of CM or leafrollers. It is also notable that few benefits were found from using higher product rates.

Using lower doses without sacrificing efficacy may make products more economically viable. Future work at the Yakima laboratory will focus on optimizing the application rate and frequency of CpGV applications as well as improving its persistence and uptake through formulation.

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References

- Ballard, J., Ellis, D.J., Payne, C.C., 2000. Uptake of granulovirus from the surface of apples and leaves by first instar larvae of the codling moth *Cydia pomonella* L. (Lepidoptera: Olethreutidae). *Biocontrol Sci. Technol.* 10, 617–625.
- Barnes, M.M., 1991. Tortricids in pome and stone fruits, codling moth occurrence, host race formation and damage. In: van der Geest, L.P.S., Evenhuis, H.H. (Eds.), *Tortricid Pests, Their Biology, Natural Enemies and Control*. Elsevier Science, Amsterdam, The Netherlands, pp. 313–327.
- Beers, E.H., Brunner, J.F., Willett, M.J., Warner, G.M., 1993. *Orchard Pest Management: A Resource Book for the Pacific Northwest*. Good Fruit Grower, Yakima, WA.
- Calkins, C.O., 1998. Review of the codling moth area wide suppression program in the western United States. *J. Agric. Entomol.* 15, 327–333.
- Cross, J.V., Solomon, M.G., Chandler, D., Jarrett, P., Richardson, P.N., Winstanley, D., Bathon, H., Huber, J., Keller, B., Langenbruch, G.A., Zimmermann, G., 1999. Biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. *Biocontrol Sci. Technol.* 9, 125–149.
- Dunley, J.E., Welter, S.C., 2000. Correlated insecticide cross-resistance in azinphosmethyl resistant codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* 93, 955–962.
- Falcon, L.A., Huber, J., 1991. Biological control of the codling moth. In: van der Geest, L.P.S., Evenhuis, H.H. (Eds.), *Tortricid Pests, Their Biology, Natural Enemies and Control*. Elsevier Science, Amsterdam, The Netherlands, pp. 355–369.
- Federici, B.A., 1997. Baculovirus pathogenesis. In: Miller, L.K. (Ed.), *The Baculoviruses*. Plenum Press, New York, pp. 33–59.
- Glen, D.M., Clark, J., 1985. Death of *Cydia pomonella* larvae and damage to apple fruit, after field application of codling moth granulosis virus. *Entomol. Exp. Appl.* 38, 93–96.
- Glen, D.M., Payne, C.C., 1984. Production and field evaluation of codling moth granulosis virus for control of *Cydia pomonella* in the United Kingdom. *Ann. Appl. Biol.* 104, 87–98.
- Gröner, A., 1986. Specificity and safety of baculoviruses. In: Granados, R.R., Federici, B.A. (Eds.), *The Biology of Baculoviruses Vol. I, Biological Properties and Molecular Biology*. CRC Press, Boca Raton, FL, pp. 177–202.
- Guillon, M., Biache, G., 1995. IPM strategies for control of codling moth (*Cydia pomonella* L.) (Lepidoptera Olethreutidae) interest of CmGV for long term biological control of pests complex in orchards. *Med. Fac. Landbouww. Univ. Gent.* 60, 695–705.
- Huber, J., 1980. Field persistence of the codling moth granulosis virus *Laspeyresia pomonella*. *IOBC/WPRS Bull.* 3, 58–59.
- Huber, J., 1986. Use of baculoviruses in pest management programs. In: Granados, R.R., Federici, B.A. (Eds.), *The Biology of Baculoviruses Vol. II, Practical Application for Insect Control*. CRC Press, Boca Raton, FL, pp. 181–202.
- Jackson, D.M., 1979. Codling moth egg distribution on unmanaged apple trees. *Ann. Entomol. Soc. Am.* 72, 361–368.
- Jakes, R.P., 1985. Stability of insect viruses in the environment. In: Maramorosch, K., Sherman, K.E. (Eds.), *Viral Insecticides for Biological Control*. Academic Press, New York, pp. 285–360.
- Jakes, R.P., 1990. Effectiveness of the granulosis virus of the codling moth in orchard trials in Canada. In: *Proceedings of the V International Colloquium on Invertebrate Pathology and Microbial Control*, Adelaide, Australia, 20–24 August 1990, pp. 428–430.
- Jakes, R., Hardman, J., Laing, J., Smith, R., 1994. Orchard trials in Canada on control of *Cydia pomonella* (LEP: Tortricidae) by granulosis virus. *Entomophaga* 39, 281–292.
- Jakes, R.P., Laing, J.E., Laing, D.R., Yu, D.S.K., 1987. Effectiveness and persistence of the granulosis virus of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae) on apple. *Can. Entomol.* 119, 1063–1067.
- Keller, S., 1973. Microbiological control of the codling moth (*Laspeyresia pomonella* (L.)) (= *Carpocapsa pomonella*) with specific granulosis virus. *Z. Ang. Entomol.* 73, 137–181.
- Knight, A.L., Potting, R.P., Light, D., 2002. Modeling the impact of a sex pheromone/kairomone attracticide for management of codling moth (*Cydia pomonella*). *Acta Hort.* 584, 215–220.
- Lacey, L.A., Shapiro-Ilan, D.L., 2003. The potential role for microbial control of orchard insect pests in sustainable agriculture. *J. Food Agric. Environ.* 1, 326–331.
- Lacey, L.A., Knight, A.L., Huber, J., 2000. Microbial control of lepidopteran pests of apple orchards. In: Lacey, L.A., Kaya, H.K. (Eds.), *Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests*. Kluwer Academic Publishers, Dordrecht, pp. 557–576.
- Lacey, L.A., Vail, P.V., Hoffmann, D.F., 2002. Comparative activity of baculoviruses against the codling moth *Cydia pomonella* and three other tortricid pests of tree fruit. *J. Invertebr. Pathol.* 80, 64–68.
- Ryer, A., 1997. *Light Measurement Handbook*. International Light, Newbury Port, MA.
- Sauphanor, B., Brosse, V., Bouvier, J.C., Speich, P., Micoud, A., Martinet, C., 2000. Monitoring resistance to diflubenzuron and deltamethrin in French codling moth populations (*Cydia pomonella*). *Pest Manag. Sci.* 56, 74–82.
- Summerland, S.A., Steiner, L.F., 1943. Codling moth oviposition and fate of eggs. *J. Econ. Entomol.* 36, 72–75.
- Toba, H.H., Howell, J.F., 1991. An improved system for mass-rearing codling moths. *J. Entomol. Soc. B.C.* 88, 22–27.
- Tukey, L.D., 1981. Growth and development in tree fruits. In: Tukey, R.B., Williams, M.U. (Eds.), *Tree Fruit Growth Regulators and Chemical Thinning*. Washington State University, Pullman, WA, pp. 1–45.
- Vail, P.V., Barnett, W., Cowan, D.C., Sibbett, S., Beede, R., Tebbets, J.S., 1991. Codling moth (Lepidoptera: Tortricidae) control on commercial walnuts with a granulosis virus. *J. Econ. Entomol.* 84, 1448–1453.

Varela, L.G., Welter, S.C., Jones, V.P., Brunner, J.F., Riedl, H., 1993. Monitoring and characterization of insecticide resistance in codling moth (Lepidoptera: Tortricidae) in four western states. *J. Econ. Entomol.* 86, 1–10.

Vickers, R.A., Rothschild, G.H.L., 1991. Use of sex pheromones for control of codling moth. In: van der Geest, L.P.S., Evenhuis, H.H. (Eds.), *Tortricid Pests, Their Biology, Natural Enemies and Control*. Elsevier Science, Amsterdam, The Netherlands, pp. 339–354.